

of the formation of the central substrate-binding site and the two sodium ion-binding sites. The sequential conformational changes from outward-open to closed state provide us with further insights into the nature of the coupled binding of trimethylammonium substrates and the two sodium ions to BetP and consequently their impact on the state transitions in the alternating-access cycle.

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The Betaine Transporter BetP - Analysis of Osmotic Stimuli Responsible for Activation

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The Na⁺ coupled betaine uptake system BetP of *Corynebacterium glutamicum* is an established model system for osmoregulated membrane transporters in bacteria. It belongs to the BCCT family of transporters and comprises both a catalytic function (betaine/Na⁺ cotransport) and a sensory/regulatory function (responding to osmotic stress).

Its 2D (electron crystallography) and 3D structure (X-ray crystallography) has been solved. Within a homooligomeric trimer, each BetP protomer harbours both an N- and a C-terminal domain involved in stimulus sensing and intramolecular signal transduction. Factors known so far contributing to the sensory and regulatory function of BetP are (i) the two terminal domains, (ii) K⁺ ions as an osmotic stress related stimulus, and (iii) interaction with the surrounding membrane. The analysis of relevant stimuli related to osmotic activation was previously done mainly in proteoliposomes (reconstituted system).

We have now performed a stimulus analysis of BetP in intact cells under different levels of internal K⁺ and found that, different from the previous conception, the second stimulus is more relevant for BetP activation in response to hyperosmotic stress under physiological conditions. This second stimulus was shown to act on BetP directly via the membrane surrounding. In addition, these results shed new light on differences between an in vivo (intact cells) and in vitro analysis (proteoliposomes) of a well-studied membrane transporter.

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Lock and Load: Key Role of the Unique Closed State in Transport Regulation of the Sodium-Coupled Betaine Transporter BetP

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The Na⁺-coupled betaine symporter BetP is regulated by osmotic stress sensing the increasing internal K⁺ concentration as a direct consequence of hypertonicity by its positively charged C-terminal domain. Recently, we demonstrated that BetP binds K⁺ cooperatively to its cytoplasmic side between different C-terminal domains within the BetP trimer, while the monomer is not regulated. K⁺ binding strengthens the helical fold of the C-terminal domain, and together with negatively charged lipids at the trimer center orients it towards the counterclockwise adjacent protomer. The extended C-terminal conformation is assisted by an additional interaction with the N-terminal domain and most crucially with lipids. A recent crystal structure shows BetP in a closed state with the C-terminal domain bending back towards its own protomer to interact mainly with lipid head groups at the membrane surface. Thereby, an intratrimeric interaction is inhibited, which allows us to assign the bend-back conformation of the C-terminal domain as the inactive one and the extended conformation as active. A surprising consequence of the inactive C-terminal conformation is the uncoupling of the two sodium sites. Although having all substrates bound BetP cannot any longer isomerize to the inward-facing state because the C-terminal domain together with lipids lock the intracellular gate preventing the opening of the S1 site. Subsequently, all BetP protomers within a trimer remain fully substrate loaded in a closed state with additional betaine molecules sequestered and stored in the second periplasmic (S2) binding site. We propose a trimeric switch model, in which osmotic stress induces a stepwise activation of one protomer after the other by consecutive K⁺ binding and straightening of the C-terminal helix.

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Complete Mapping of Substrate Translocation Implicates the Secondary Binding Site and Highlights the Significance of LeuT N-Terminal Segment in Regulating Transport Cycle

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Neurotransmitter:sodium symporters (NSSs) regulate neurotransmission by clearing excess neurotransmitters from the synaptic cleft, assisted by

co-transport of sodium ions. Crystal structures resolved for a prokaryotic orthologue LeuT opened the way to structure-based studies in search of a mechanistic understanding of substrate transport by NSSs. Yet, this goal has been elusive due to the complex interplay of global and local events as well as missing structural data on LeuT N-terminal segment implicated in intracellular gating. We have extended our recent study,¹ to obtain for the first time a comprehensive time-resolved mechanistic description of the complete transport cycle, using a combination of conventional and advanced molecular dynamics simulations. Our simulations suggest that LeuT harbors two substrate-binding sites for alanine. In the outward-facing open state, binding of substrate (and sodium ions) to the primary-site S1 regulates subsequent redistribution of molecular interactions to trigger extracellular gate closure; whereas the secondary-site S2 is only a transient binding site. Substrate-binding affinity at S2 increases in an intermediate close to inward-facing state. Small displacements in the second substrate near S2 are observed to induce concerted small translocations in the substrate bound to S1, although complete release requires collective structural rearrangements that fully expose the intracellular vestibule to the cytoplasm. Redistribution of salt bridges and cation- π interactions involving the N-terminal segment residues plays a pivotal role in mediating substrate release and closure of intracellular gate, and triggering a global reconfiguration to resume the transport cycle.

Reference:

1. Cheng MH, Bahar I (2013) Coupled Global and Local Changes Direct Substrate Translocation by Neurotransmitter-Sodium Symporter Ortholog LeuT *Biophys J* 105: 630-639.

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Analyzing a Conformational Sampling of LeuT from Accelerated Molecular Dynamics Simulations

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Monoamine transporters are a key feature in controlling interneuron communication by facilitating the reuptake of neurotransmitters such as dopamine, serotonin, and norepinephrine. These reuptake transporters have been computationally studied primarily through the use of homology modeling to the structurally related bacterial LeuT structure or by direct simulations of LeuT. It is thought that finding the different conformations of LeuT will aid in elucidating the mechanism of transport. To this end, a conformational sampling of LeuT was done by performing accelerated molecular dynamics (aMD) simulations on a biological membrane and an embedded LeuT transporter with different combinations of the leucine substrate and bound sodium ions. Conformational clusters were found by applying principal component analysis resulting in seven distinct structures. Both the seven structures and the entire trajectories were studied by looking at specific residues, transmembrane helical domain positions and interactions, and solvent accessibility.

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Dopamine Transporter Inhibition by Organic Ions & their Effects on GBR12909 Inhibition

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Dopamine transporters (DATs) in the presynaptic neuron uptake the remaining dopamine in the synaptic cleft, thereby terminating signal transmission and maintaining presynaptic dopamine storage. DATs co-transport two Na⁺ and one Cl⁻ with one dopamine; the driving force for dopamine uptake is the concentration gradients of Na⁺ and Cl⁻. Consequently, dopamine uptake does not occur when either Na⁺ or Cl⁻ is not present. Interestingly, however, other inorganic ions act like DAT inhibitors, suggesting they would compete with Na⁺ or Cl⁻ binding, thus reducing the driving force for dopamine transport. Some organic ions are also known to inhibit dopamine uptake while the underlying mechanism is not yet clear. Here, human DAT (hDAT) inhibition by organic cations (NMDG and choline) and anions (gluconate and isethionate) was studied using fluorescence-based dopamine uptake assays. All tested organic ions inhibited hDAT in a concentration-dependent manner, exhibiting complex inhibitory effects associated with their size and charge (IC₅₀ values ranging from 1 to 37 mM). In particular, NMDG showed a stiff sigmoidal curve with an apparent Hill coefficient (*h*) of 11 while other ions have *h* values of 1, suggesting hDAT may have multiple binding sites for NMDG. To investigate further the inhibition mechanism by organic ions,